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Minireview

Rapid validation of molecular structures of biological samples by electrospray-mass spectrometry

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Abstract

A short account is presented of the method of measuring molecular masses (M_r) of pure biological samples by electrospray ionisation mass spectrometry. It is demonstrated that the technique yields M_r values with an effective accuracy equal to or better than 0.008% of the calculated M_r, provided that the correct molecular structure is employed in the calculation. It is therefore recommended that this method of measuring M_r's should be considered to form an essential part of all studies aimed at elucidating the molecular structure of purified biological macromolecules or for confirming the identity of labelled samples of such molecules.

Key words: Molecular mass; Electrospray ionisation mass spectrometry; Effective accuracy; Protein; Glycoprotein

1. Introduction

The powerful technique of mass spectrometry (MS) requires the formation of ions from the analyte as the essential first step for each and every analysis. Until the mid-1970–1980's, MS analysis had generally been confined to volatile, neutral, low molecular weight organic molecules ($M_r \leq 300$ Da) because the ionisation method employed, electron impact (EI), was only suitable for gases or vapours. The resulting molecular ions readily fragmented on account of the excess energy imparted to them during the ionisation process, to yield complex EI-MS spectra which could be interpreted in terms of molecular structure [1]. Then, two new 'soft' or low energy ionisation methods were discovered which were based upon the desorption of the molecular ions of non-volatile analytes already present in solid or liquid matrices into the gas phase, namely fast atom bombardment (FAB-MS) [2] and ^{252}Cf plasma desorption (PD-MS) [3] methods. These ionisation procedures initiated the transformation of organic mass spectrometry towards biological mass spectrometry since they enabled the analysis of non-volatile, polar and relatively large biomolecules to be made routinely. By 1987, one of us [4] had successfully ionised a protein of M_r = 24,000 Da by FAB-MS and by 1989 Roepstorff's group in Denmark reported the ionisation of a protein with M_r = 45,000 Da by PD-MS [5]. However, it was the publication in 1988 of the details of two other new, soft ionisation techniques, electrospray ionisation (ESI) [6] and matrix assisted laser desorption ionisation (MALDI) [7], both of which could produce ions from very large, polar, non-volatile molecules (M_r = 200–300 kDa) which completed the transformation. These initial reports generated such intense interest and worldwide practical activity that by 1990, Burlingame et al. [8] were able to state confidently in their biannual review of mass spectrometry that, 'MS methods may now be applied to virtually any structural biology problem', and to predict that such methods, 'are likely to become the method of choice for many biological studies'.

In the present minireview, we summarise some of our experiences of ESI-MS gained during the last 4 years on commercial triple quadrupole MS instruments (VG BioQ). In particular, we shall focus our attention on a discussion of the excellent effective accuracy of M_r measurements for pure samples made using this technique and the implications of this for structural studies of biomolecules. For major, comprehensive reviews of the literature on biological MS the reader is referred to the recent publications of Burlingame et al. [9] and Biemann [10].

2. Principles and practice of ESI-MS

Electrospray ionisation (ESI) is a method of produc-

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ing singly or multiply charged molecular ions from an analyte solution by spraying it (the solution) under the influence of a strong electrical field. The fine spray of solution droplets (d_p = 1.2 μm) is formed by allowing the solution, flowing at 2–20 μl/min, to emerge from the tip of a very fine stainless-steel (SS) capillary, which is encased in a wider bore SS capillary through which a stream of warm N_2 gas is maintained, and from which it protrudes some 0.5 mm. For positive ion formation a voltage of + 3.5 kV is applied to the SS capillary; for negative ion formation the polarity of the applied voltage is reversed. The solvent is removed from the electrospray droplets by the nitrogen gas, leaving the analyte molecular ions in the gas phase at atmospheric pressure ready for entry into the reduced pressure regions of the mass spectrometer via a pair of sampling orifices. Whilst the exact mechanism by which the gaseous analyte molecular ions are formed is still not known [11], in practice this simple ionisation procedure works well. The fact that ESI yields stable *multiply*-charged molecular ions from solutions of macro polyelectrolytes such as proteins is of crucial practical importance because it enables the use of relatively small spectrometers, e.g. with a maximum *m/z* range of 3,000 for singly charged ions, to measure large M_r's. Thus the molecular ion of a protein of M_r = 100,000 Da with 100 added protons would appear at an *m/z* ratio of 100,100/100 or 1001.0. Most acidic protein solutions produce a series of multiply charged molecular ions with each ion in the series differing by ± 1 proton from adjacent ions in the series, as shown in Fig. 1a (for horse heart myoglobin). A mass spectrometer measures the mass-to-charge ratio (*m/z*) of each ion peak so that generally:

$$(m/z) = \frac{M_r + n\mathrm{H}}{n}$$

where H = proton mass = 1.00794 Da and n = number of protons (charges) for any particular ion peak. Hence:

$$M_r = n\,((m/z) - \mathrm{H}) \quad (1)$$

To determine n, any two consecutive peaks differing by one proton in the series may be used as follows with reference to Fig. 1a.

$$(m/z)_2 = \frac{M_r + n\mathrm{H}}{n} \quad \text{and}$$

$$(m/z)_1 = \frac{M_r + (n+1)\mathrm{H}}{n+1} \quad \text{so that}$$

$$n = \frac{(m/z)_1 - \mathrm{H}}{(m/z)_2 - (m/z)_1} \quad (2)$$

To aid interpretation, electrospray spectra are normally transformed by routine data system procedures which utilise Eqs. 1 and 2 [12] so that all of the ion peaks in the original spectrum (Fig. 1a) originating from one protein are combined and presented as a single peak on a true M_r scale, as shown in Fig. 1b. The M_r's of proteins measured by MS are chemical average values based on the average atomic weights of the elements. Mixtures of proteins produce even more complex spectra since each component gives rise to its own series of multiply charged peaks which do not interact [13] even though they may sometimes fortuitously overlap.

In our laboratories, samples of the purified, de-salted protein or glycoproteins studied are generally made up into aqueous acetonitrile solutions (50/50 v/v)/1% formic acid, of final concentration 25–50 pmol/μl. Generally, a 10 μl aliquot of the analyte solution is injected, via a loop injector, into a stream of the same solvent mixture flowing at a rate of 5 μl/min. The mass spectrometer is then routinely scanned over an appropriate *m/z* range, deter-

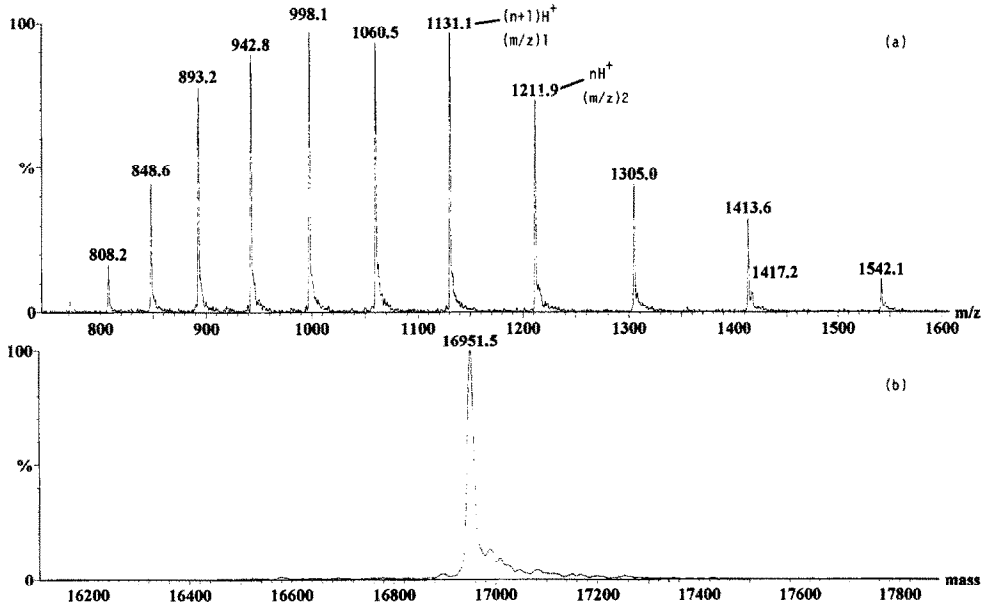

Fig. 1.

mined experimentally, generally 10 s scans, and several scans (say ≤ 15) are summed to obtain the final ESI-MS spectrum. Calibration of the *m/z* scale of the spectrometer is normally performed during each series of measurements using a solution of pure protein of known structure.

3. Validation of published macromolecular structures

At the present time two complementary experimental methods are employed to determine the three dimensional molecular structure of biological macromolecules, namely X-ray crystallography and nuclear magnetic resonance spectroscopy. Unfortunately, mistakes can and do sometimes occur in the collection and interpretation of the data generated by these techniques [14], and in the determination of the backbone structure (primary sequence in the case of proteins) used for the initial interpretation of the former data [15]. In addition to these well documented sources of error, it should be noted that very few samples of the biological macromolecules for which a 3D structure has been determined appear to have been assayed for purity prior to their structural analysis so that possible errors of interpretation of the experimental data due to the presence of impurities may also occur. The publication and dissemination of erroneous molecular structures in computerised data banks is a matter of serious concern since they form the basis of all subsequent studies on the function of the biological macromolecules involved and of any attempted alteration of that function by protein engineering or drug design. Therefore, the primary aim of the present minireview is to draw attention to the fact that it is now possible to routinely verify published macromolecular structural data by ESI-MS. It will now be shown that these measurements, which can be quickly made in some 2–15 mins, yield such accurate M_r's that the published structures can either be confirmed or disproved. Further it will be demonstrated that in the case of the latter finding, calculation of the exact magnitude of the mass difference between the measured M_r and the published M_r can often be used to indicate the origin of the discrepancy so as to aid further studies aimed at resolving the conflicting data. Thus Table 1 below summarises our ESI-MS measurements of M_r for pure samples of just three macromolecules, which were chosen from data obtained for many hundreds of samples because two different structures had been published for them.

The first protein listed in Table 1, horse heart myoglobin, illustrates the power of the ESI-MS technique to detect minor errors in protein sequence data. As indicated in Fig. 1, horse heart myoglobin is used as a molecular mass standard primarily because of its commercial availability, purity and lack of glycosylation. When it was first used as a calibrant by us in 1989 the second calculated M_r value of 16,950.5 Da given in Table 1 was employed because this value corresponded to the published sequence data given in the primary literature [16] which had been abstracted in the Atlas of Protein Sequence and Structure [17]. However, by the autumn of 1990 when we were engaged upon a study of abnormal haemoglobins, certain systematic errors were found and so we decided to measure the M_r of myoglobin itself using a mixture of normal α- and β-globin as calibrants. When this was done the $M_{r(meas)}$ = 16,951.5 Da listed in Table 1 was obtained from a total of eight distinct measurements. Because of the vast amount of work which has

Table 1
The measured mean and calculated molecular masses of some biological macromolecules having two reported structures, together with the corresponding mass discrepancy (D) and effective accuracy (EA%) of the measurements

Name	$M_{r(meas)}$ Da (*n*)	S.D. (Da)	S.E.M. (Da)	Molecular formula					$M_{r(calc)}$[a] (Da)	D[b] (Da)	S.D. × 100 / $M_{r(calc)}$	EA[c] (%)
				C	H	N	O	S				
Myoglobin (horse heart)	16,951.5 (8)	0.16	0.06	769	1,212	210	218	2	16,951.5	0.0	0.0009	0.001
Myoglobin-H				769	1,211	210	218	2	16,950.5	1.0	0.0009	0.007
Retinol binding protein (RBP)	21,064.8 (5)	0.67	0.30	926	1,410	260	285	10	21,065.6	−0.8	0.0032	0.008
RBP-Leu				920	1,399	259	284	10	20,952.5	112.3	0.0032	0.542
α-Fetoprotein (AFP)	68,799.2 (7)	3.80	1.44	3,012	4,730	796	964	40	68,800.1	−0.9	0.0055	0.008
AFP-Arg				3,006	4,718	792	963	40	68,643.9	155.3	0.0055	0.232

S.E.M., standard error in the mass mean ($M_{r(meas)}$); S.D. is the standard deviation of the measured M_r values; *n* is the number of distinct (and recalibrated) measurements.

[a] The $M_{r(calc)}$ values were obtained from the computed molecular formulae, derived from the structures given in the corresponding reference, in the text, and have been rounded to 1 decimal place. The following average atomic masses were employed: C = 12.011; H = 1.00794; N = 14.00674; O = 15.9994; S = 32.066 (see [(1991) Pure Appl. Chem., 63, 975–990]).

[b] $D = M_{r(meas)} - M_{r(calc)}$.

[c] $EA\% = [\,|\,3 \times S.E.M.\,| + |\,D\,|\,] \times 100/M_{r(calc)}$ where $S.E.M. = \frac{S.D.}{\sqrt{n}}$.

been performed on the globins [18] we were convinced of the validity of their sequences and hence of their calculated M_r's, and therefore we concluded that the 1969 published and 1972 abstracted sequence for myoglobin was incorrect. Further, consideration of the magnitude of this mass difference of 1 Da led us to propose that either one aspartic or one glutamic acid residue had been incorrectly identified. The first of these proposals was subsequently confirmed by a manual literature search which revealed that Lehmann's group at Cambridge had re-assigned the asparagine residue at 122 to aspartic acid, first in 1974 [19] and again in 1975 [20] when they compared the sequences of myoglobin from horse and zebra. Accordingly since late 1990, whenever we have used myoglobin (horse heart) as a calibrant we have always employed the experimentally determined and calculated corrected sequence value of M_r = 16,951.5 Da [21]. It should perhaps be noted that this sequence had been adopted by the group which determined the 3D structure of horse heart myoglobin using X-ray crystallographic techniques [22]. Additional evidence in support of the correctness of our experimental mean M_r value comes from a recent paper [23] which reports a combined enzymatic hydrolysis/FAB-MS determination of the sequence of the relevant peptide (119–133) from horse heart myoglobin.

The second protein listed, human serum retinol binding protein (RPB), affords an example of the use of ESI-MS to identify major errors in published protein sequences, i.e. errors in the length of the primary sequence. The M_r of RBP was routinely measured and, as shown in Table 1, was found to be 21,064.8 Da. In this case we first compared the measured M_r with that calculated from the reported primary sequence of 182 residues [24] because it was employed by the Uppsala group of crystallographers for their 3D structural determination [25]. As shown in Table 1, the corresponding calculated M_r = 20,952.5 Da and hence the difference between this and the experimental value is 112.3 Da, indicating that the published protein sequence was probably lacking a leucine or an isoleucine residue (113.2 Da) or possibly an asparagine residue [114.1 Da). A comprehensive literature search was then undertaken and this revealed that the Uppsala crystallographic group had later published [26] a refinement of their previous X-ray structure of RBP to 2 Å resolution using a primary sequence of 183 residues with an additional leucine residue at the C-terminal albeit without comment or reference! Clearly this new sequence confirms the validity of our experimental mean M_r value listed in Table 1 and indicates that the most plausible of our suggestions, namely an additional leucine (or isoleucine), was in fact correct.

Human α-fetoprotein (AFP), the third illustrative example of a biological macromolecule with two reported protein structures listed in Table 1 is, in spite of its name, a glycoprotein. A study of the measured mean M_r value obtained for the sample of AFP employed and comparison with that calculated using the published structure of the major glycan [27] and the most recent (1991) of the reported structures of the protein [18] shows excellent agreement. However, if the mass of this glycan is added to the calculated mass of the first (1983) reported structure of the protein [29] a calculated M_r = 68,643.93 Da results, reflecting the mass difference due to the 'missing' N terminal arginine residue in this structure. Such a large mass difference would have been immediately apparent from the present ESI-MS measurements and a confident prediction of a missing arginine residue would have been possible had only this first protein structure been published. It should perhaps be noted that the (AFP) glycoprotein example presented here was chosen because it exists mainly as a single glycoform [30]. The majority of glycoproteins which we have analysed to date exist as complex (and variable) mixtures of different glycoforms the structures of which may [31] or may not [32] be resolved by ES-MS techniques alone.

Thus far in this discussion of the M_r data no reference has been made to the effective accuracy (EA%) figures forming the last column of Table 1. The accuracy of $M_{r(meas)}$ achieved by ESI-MS has been stated [33] to be 'generally to within 0.005% of the calculated value' when the correct structure is used in the calculation. However, it is not clear to us, or to others, if this statement was based upon consideration of single or repeat $M_{r(meas)}$. Thus Biemann [10] states that 'the relatively high accuracy, or more precisely speaking, the precision of the molecular mass measurement by ESI-MS, is chiefly the result of averaging individual measurements in the same spectrum'. In an attempt to clarify the situation, and since all of the mass measurements reported here were repeat measurements (4- to 8-fold replication) performed on the same or different samples, we decided to calculate the EA- value of the measurements. The definition of this measure of accuracy is given in the legend of Table 1. We believe that it (EA) provides a realistic error estimate for the method, provided n is sufficiently large, since it incorporates possible scale bias and statistical error in the mean for the selected degree of replication, and it will be seen (Table 1, column 8) that for the three biomolecules studied the maximum value of EA is 0.008%. Therefore the above statement concerning the accuracy of ESI-MS measurements should be modified to include this value of EA if comparisons are being made using the mean value of repeat measurements of M_r. Thus for a pure biomolecule of $M_{r(calc)}$ = 10,000 Da the *maximum* experimental error associated with repeat determinations of its M_r would be ± 0.8 Da; for a pure biomolecule of $M_{r(calc)}$ = 100,000 Da the *maximum* experimental error associated with repeat $M_{r(meas)}$ would be ± 8.0 Da provided horse heart myoglobin or a calibrant of equivalent qualities could be used. Comparison of these maximum error estimates of 0.008% with those of ± 20% com-

monly encountered with current SDS-PAGE methods of M_r determination illustrate the transformation which ESI-MS has brought to the accuracy of the measurement of this key physico-chemical property of a biomolecule. In view of this we should like to recommend that in future, ESI-MS measurement of M_r should be considered to form an essential part of all studies aimed at elucidating the molecular structure of biological macromolecules. In particular, since we have measured M_r of a single protein crystal which had been previously used in X-ray crystallographic studies [34], there is no practical reason for omitting accurate M_r measurements using ESI-MS on such crystalline samples in order to check the validity of their amino acid sequences as determined by classical chemical or molecular biological methods.

To conclude this discussion of the general applicability of our EA measurements we would like to re-emphasise the fact that these were made on pure, homogenous, de-salted samples of the biomolecules using a carefully calibrated and re-calibrated quadrupole mass spectrometer. All users, and potential users, of the ESI-MS technique need to be aware of the influence of the purity of their samples upon the validity of the M_r results. Thus, if the sample is contaminated with peptides or other proteins then an ESI-mass spectrum can generally be obtained which, in many cases, can be interpreted in terms of the molecular components of the mixture (see e.g. [21–35]. However, if the sample is heavily contaminated with salts, or non-volatile buffers, then no ESI-MS spectrum can be obtained. If, on the other hand, only traces of salts are present in the samples, then ESI-MS spectra may be obtained of the ionic adducts of the proteins and of any free proteins. Unfortunately, it is somewhat fortuitous if the corresponding molecular ion peaks of the adducted and non-adducted species are resolved [36] or not. Clearly, if they are not completely resolved then the measured M_r will be erroneous. The solution to this practical problem is thus to ensure that all samples submitted for ESI-MS analysis have been vigorously desalted, a process which may be accomplished by standard dialysis, ultrafiltration or by liquid chromatographic procedures. It may be of interest to note here that one of us [37] has found that ESI-MS is tolerant to the presence of small amounts of PEG (and probably by analogy to PPG) in protein samples which had been incompletely purified by dialysis. Finally on the subject of sample purity, it should be noted that the authors of the recent important paper [38] reporting accurate M_r measurements on samples of complex mixtures of proteins, which had been separated on SDS-PAGE gels, followed by electroelution, also emphasised the need for rigourous removal of salts and SDS before the ESI-MS measurements were attempted.

Further, we should also like to draw attention to the ability of the method to ascertain whether commercial (or academic) samples of biomolecules have been correctly labelled. If a sample has been mislabelled then the mass discrepancy value $D = M_{r(meas)} - [M_{r(calc)}]_{label}$ may assume any value according to the molecular structures of the two biomolecules involved. If $D = 0$ Da, then the ESI-MS measurements cannot assist, but in all other cases provided $D \times 100[M_{r(calc)}]_{label} > EA$, then the mass spectrometric method would indicate a discrepancy. Up to the present time we have only encountered one case of mislabelling [35], that of δ-chymotrypsin (M_r = 25,430.9 Da) which had been mislabelled as α-chymotrypsin (M_r = 25,233.7 Da). However, cases have been reported by other workers [39], for example of porcine β-trypsin (M_r = 23,463.6 Da) being labelled as bovine β-trypsin (M_r = 23,293.31 Da). It is thus clearly necessary and now feasible using ESI-MS methods to check the identity of labelled samples since the current practice of providing, say, a UV spectrum, an HPLC chromatogram, a CZE electropherogram, amino acid analysis, biological activity data, enzymic rate constants, is now known to be insufficient to characterise a biological material [40].

The important applications of MS presented and discussed in this minireview all stem from the ability of ESI to produce a series of multiply charged molecular ions. It remains to be seen if the experiments which are now underway in many laboratories, including our own, aimed at fragmenting these molecular ions and then measuring and analysing the multiply charged fragment ions so as to determine the amino acid sequence of large biomolecules, will prove successful on a routine basis.

References

[1] McClafferty, F.W. and Turecek, F. (1993) Interpretation of Mass Spectra, 4th Edn., Freeman/University Science Books.
[2] Barber, M., Bordoli, R.S., Sedgwick, R.D. and Tyler, A.N. (1981) J. Chem. Soc. Chem. Commun. 325–371.
[3] Torgerson, D.F., Skowronski, R.P. and Macfarlane, R.D. (1974) Biochem. Biophys. Res. Commun. 60, 616–624.
[4] Barber, M. and Green, B.N. (1987) Rapid Commun. Mass Spec. 1, 80–83.
[5] Jonsson, G., Hedin, A., Hakansson, P., Sundquist, B.U.R., Bennich, H. and Roepstorff, P. (1989) Rapid Commun. Mass Spec. 3, 190–191.
[6] Meng, C.K., Mann, M. and Fenn, J.B. (1988) Z. Phys. D. 10, 361–368.
[7] Karas, M. and Hillenkamp, F. (1988) Anal. Chem. 60, 2299–2301.
[8] Burlingame, A.L., Millington, D.S., Norwood, D.L. and Russell, D.H. (1990) Anal. Chem. 62, 268R–303R.
[9] Burlingame, A.L., Baillie, T.A. and Russell, D.H. (1992) Anal. Chem. 64, 467R–502R.
[10] Biemann, K. (1992) Annu. Rev. Biochem. 61, 977–1010.
[11] Ashton, D.S., Beddell, C.R., Cooper, D.J., Green, B.N. and Oliver, R.W.A. (1993) Org. Mass Spec. 28, 721–728.
[12] Mann, M., Meng, C.K. and Fenn, J.B. (1989) Anal. Chem. 61, 1702–1708.
[13] Green, B.N. (1992) Biochem. J. 284, 603–604.
[14] Bränden, C.I. and Jones, T.A. (1990) Nature 343, 687–689.

[15] Gibson, B.W. and Biemann, K. (1984) Proc. Natl. Acad. Sci. USA 81, 1956–1960.
[16] Dautrevaux, M., Boulanger, Y., Han, K. and Biserte, G. (1969) Eur. J. Biochem. 11, 267–277.
[17] Dayhoff, M.O. and Eck, V. (1972) Atlas of Protein Sequence and Structure, vol. 5, pp. D-82.
[18] Bunn, H.F. and Forget, B.G. (1986) Hemoglobin: Molecular, Genetic and Clinical Aspects, Saunders, Philadelphia.
[19] Romero-Herrara, A.E. and Lehmann, H. (1974) Biochim. Biophys. Acta 336, 318–323.
[20] Romera-Herrara, A.E. and Lehmann, H. (1975) Biochim. Biophys. Acta 393, 201–204.
[21] Oliver, R.W.A. and Green, B.N. (1991) Trends Anal. Chem. 10, 85–91.
[22] Evans, S.V. and Brayer, G.D. (1988) J. Biol. Chem. 263, 4263–4268.
[23] Zaia, J., Annan, R.S. and Biemann, K. (1992) Rapid Commun. Mass Spec. 6, 32–36.
[24] Rask, L., Anundi, H. and Peterson, P.A. (1979) FEBS Lett. 104, 55–58.
[25] Newcomer, M.E., Jones, T.A., Aqvist, J., Sundelin, J., Eriksson, U., Rask, T. and Peterson, P.A. (1984) EMBO J. 3, 1451–1454.
[26] Cowan, S.W., Newcomer, R.E. and Jones, T.A. (1990) Proteins 8, 44–61.
[27] Terrana, B., Tecce, M.F., Manetti, R., Ceccarini, C., Lamba, D. and Segre, A.L. (1990) Clin. Chem. 36, 879–882.
[28] Pucci, P., Siciliano, R., Malorni, A., Marino, G., Tecce, M.F., Ceccarini, C. and Terrana, B. (1991) Biochemistry 30, 879–882.
[29] Moringa, T., Sakai, M., Wegmann, T.G. and Tamoki, T. (1983) Proc. Natl. Acad. Sci. USA 80, 4604–4608.
[30] Tarelli, E., Ashcroft, A.E., Calam, D.H., Corran, P.H., Green, B.N. and Wheeler, S.F. (1992) Biol. Mass Spect. 21, 315–317.
[31] Green, B.N. and Oliver, R.W.A. (1991) Trans. Biochem. Soc. 19, 939–935.
[32] Mason, A.B., Miller, M.K., Fund, W.D., Banfield, D.K., Savage, K.J., Oliver, R.W.A., Green, B.N., MacGillivray, R.T.A. and Woodworth, R.C. (1993) Biochemistry 32, 5472–5479.
[33] Smith, R.D., Loo, J.A., Edwards, C.G., Baringa, C.J. and Udseth, H.R. (1990) Anal. Chem. 62, 882–899.
[34] Wireko, F.C. and Abraham, D.J. (1991) Proc. Natl. Acad. Sci. USA 88, 2209–2211.
[35] Ashton, D.S., Beddell, C.R., Cooper, D.J., Green, B.N., Oliver, R.W.A. and Welham, K.J. (1993) Biochem. Biophys. Res. Commun. 340, 75–81.
[36] Chowdhury, S.K., Katta, V., Beavis, R.C. and Chait, B.T. (1990) J. Am. Mass Spectrom. 1, 382–388.
[37] Poulter, L.K., Green, B.N., Kaur, S. and Burlingame, A.L. (1990) in: Biological Mass Spectrometry, (A.L. Burlingame and J.A. McCloskey, Eds.), Elsevier, Amsterdam, pp. 119–128.
[38] le Maire, M., Deschamps, S., Moller, J.V., le Caer, J.P. and Rossier, J. (1993) Anal. Biochem. 214, 50–57.
[39] Vestling, M.M., Murphy, C.M. and Fenselau (1990) Anal. Chem. 62, 2391–2394.
[40] Smith, A.J., Young, J.D., Carr, S.A., Marshak, D.R., Williams, L.C. and Williams, K.R. (1992) in: Techniques in Protein Chemistry, vol. 111, pp. 219–229, Academic Press, New York.